Friedrich, T., Breiderhoff, T., and Jentsch, T.J. (1999). J. Biol. Chem. *274*, 896–902.

Grunder, S., Thiemann, A., Pusch, M., and Jentsch, T.J. (1992). Nature *360*, 759–762.

Hume, J.R., Duan, D., Collier, M.L., Yamazaki, J., and Horowitz, B. (2000). Phys. Rev. *80*, 31–81.

Jentsch, T.J., Friedrich, T., Schriever, A., and Yamada, H. (1999). Pflügers Arch. *437*, 783–795.

Kawasaki, M., Uchida, S., Monkawa, T., Miyawaki, A., Mikoshiba, K., Marumo, F., and Sasaki, S. (1994). Neuron *12*, 597–604.

Paulmichl, M., Li, Y., Wickman, K., Ackerman, M., Peralta, E., and Clapham, D.E. (1992). Nature *356*, 238–241.

Piwon, N., Gunther, W., Schwake, M., Bosl, M.R., and Jentsch, T.J. (2000). Nature *408*, 369–373.

Shimada, K., Li, X., Xu, G., Nowak, D.E., Showalter, L.A., and Weinman, S.A. (2000). Am. J. Physiol. Gastrointest. Liver Physiol. *279*, G268–G276.

Stobrawa, S.M., Breiderhoff, T., Takamori, S., Engel, D., Schweizer, M., Zdebik, A., Bösl, M.R., Ruether, K., Jahn, H., Draguhn, A., et al. (2001). Neuron *29*, this issue, 185–196.

Valverde, M.A., Diaz, M., Sepulveda, F.V., Gill, D.R., Hyde, S.C., and Higgins, C.F. (1992). Nature *355*, 830–833.

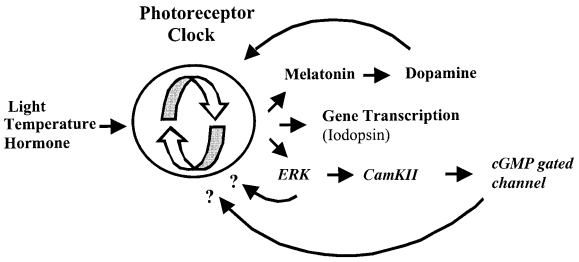
Wang, L., Chen, L., and Jacob, T.J.C. (2000). J. Physiol. *524*, 63–75.

Coupling an Activated MAP Kinase to Circadian Clock Output

Circadian rhythms, generally detected as ~24 hr oscillations in behavioral, physiological, or biochemical processes, are widespread in organisms ranging in complexity from the blue-green algae to flowering plants and man. Endogenous circadian clocks drive such rhythms. Recently, the analysis of model organisms has resulted in two major generalizations (reviewed in Dunlap, 1999). First, circadian clocks appear to be cell autonomous oscillators. Even in multicellular systems where overt physiological rhythms require intercellular signaling, rhythm generation is fundamentally a cellular process. Second, at their core circadian clocks involve self-sustained oscillations in gene expression. For example, in flies and mice, the bHLH transcriptional activators CLOCK and BMAL (also called CYCLE) drive transcription of the clock genes Period, Timeless, or Cryptochrome. The resulting proteins dimerize (in different combinations depending on the species) and inhibit CLOCK/BMAL activated transcription. This alternation of positive and negative regulation results in persistent rhythms of mRNA and protein, which represent the core oscillation.

To be physiological, relevant oscillations in clock gene expression must be entrained (synchronized) to environmental cycles such as light and must be coupled to important output pathways (see figure). The molecular details of signaling pathways coupling circadian clocks to both inputs and outputs represents fertile ground and data appear to be accumulating rapidly. A major advance for photoreceptor clocks is reported by Ko et al. (2001) in this issue of *Neuron*.

The retina has long been recognized as the site of a circadian clock that controls a wide range of local rhythms (Besharse and Iuvone, 1983). The synthesis of melatonin, rod-cone dominance, retinomotor movements, components of the electroretinogram, visual sensitivity, gene transcription, and photoreceptor membrane turnover all exhibit persistent rhythms in constant conditions (reviewed in Cahill and Besharse, 1995). Furthermore, circadian clock properties have been localized to retinal photoreceptors in both the African clawed frog (Cahill and Besharse, 1993) and chicken (Pierce et al., 1993). In the chicken, a rhythm of transcription of the cone photopigment gene (iodopsin) persists in dissociated culture under constant conditions implicating cones as clock cells. Now Ko et al., using a similar culture system, show that the Erk form of mitogen activated protein kinase and Ca^{2+}/calmodulin-dependent protein kinase II (CamKII) are part of a signaling pathway regulating a rhythm in the gating properties of the cGMP-gated channel.



Hypothetical Relationship of a Photoreceptor Circadian Clock to Input and Output Pathways

Dopamine can be regulated by melatonin and is known to directly effect the clock. Feedback of other output pathways is hypothetical.

Using excised patches from the cell bodies of embryonic photoreceptors, Ko et al. show an ~2-fold higher affinity of the cGMP-gated channel for its ligand at night. The rhythm persists for at least 2 days in constant darkness, can be entrained to light–dark cycles, and results from posttranslational modification of the channel. This is the first report of a circadian rhythm at the primary level of visual transduction, but its functional consequences for vision are not entirely clear. The photoreceptors in this analysis lack fully formed outer segments, but appear to be cones. The properties of the channel match those of mature cones, and the cultures are enriched in cones. Assuming that a similar rhythm occurs in fully differentiated cones, the measured affinity difference could result in a notable difference in the photoreceptor dark current and responsiveness to light at different times of day. It is also likely that events occurring at the level of photoreceptor transduction in cones could contribute to more complex circadian changes seen in the avian electroretinogram.

Ko et al. also demonstrate antiphasic rhythms in the phosphorylated (active) forms of Erk and CamKII with peak activities during the night and day respectively. This along with the finding that selective inhibition of the phosphorylation and activation of Erk or CamKII blocks changes in channel affinity in a manner dependent on time of day, strongly suggests that Erk and CamKII are components of a pathway that increases

channel affinity at night and decreases it during the day. Interestingly, inhibition of Erk phosphorylation also inhibits rhythmic changes in CamKII, while inhibition of CamKII phosphorylation does not affect changes in p-Erk. Based on this finding, the authors suggest that Erk is upstream of CamKII in a signaling pathway from the clock (see figure).

The simplest and most plausible interpretation is that the cultured photoreceptors are cell autonomous oscillators driving changes in Erk and CamKII to control physiology at the level of the cGMP-gated channel. A potential caveat is that the measured changes were from mixed cell cultures. Although the cultures were enriched in photoreceptors, the factors driving circadian changes in Erk and CamKII are not known and could involve intercellular signals. Within the intact retina, the diffusible modulators melatonin and dopamine, driven at least in part by circadian oscillators, play such a role, and dopamine receptors that modulate cAMP levels are found on photoreceptors (Cahill and Besharse, 1993, 1995). Molecular definition of the mechanisms that couple the "clock" to changes in Erk and CamKII phosphorylation would clarify this issue.

Like most significant contributions, that of Ko, Ko, and Dryer may prove most important in raising new questions. For example, how does Erk and CamKII activity control channel gating? Previous work has shown that the cGMP-gated channel of rods can be modified by phosphorylation (Molokanova et al., 1997) and by Ca^{2+}/calmodulin binding (Hsu and Molday, 1993). However, in the absence of direct analysis of channel phosphorylation by Erk and CamKII, it remains possible that the effects are indirect.

Likewise, the molecular mechanism coupling the clock to rhythmic changes in Erk and CamKII activity is not directly addressed. Circadian clocks often control rhythmicity through transcriptional regulation of downstream genes, and this can occur through a mechanism similar to that of CLOCK/BMAL regulated transcription of oscillating clock genes . However, Erk protein abundance does not vary during the day; it is p-Erk that is rhythmically controlled. Thus, one must look upstream of MEK1, the enzyme responsible for activating Erk, or to the phosphatases necessary for Erk dephosphorylation. It is also likely that cross-talk between Erk and other signaling pathways is involved. For example, dopamine-induced decreases in cAMP at night are known to mediate phase shifts in the core photoreceptor oscillator (Hasegawa and Cahill, 1999), and the cAMP pathway can lead to increased activation of Erk in some system.

Recent reports relating Erk activation to circadian phase shifting suggests a more general role for p-Erk in clock cells. For example, a rhythm in p-Erk similar to that reported by Ko et al. has also been seen in chick pinealocytes. Here light acutely reduces p-Erk at night, and inhibition of Erk phosphorylation appears to phase shift the clock (Sanada et al., 2000). p-Erk has also been implicated in circadian phase shifting in the mammalian suprachiasmatic nucleus (Obrietan et al., 1998). Interestingly, cross-talk with a cAMP signaling system involved in phase shifting (Ginty et al., 1993) may be of significance in the mammalian systems.

The studies on phase shifting differ from that of Ko et al. in that p-Erk is proposed to be in an "input" pathway to the clock. Although based on different systems that could use p-Erk signaling in different ways, these data raise the interesting question of whether p-Erk plays a role both as an "output" and an "input" of the clock. Recent modeling of circadian clocks suggests that rhythmic clock outputs can feedback onto the core oscillator to increase its overall stability (Roenneberg and Merrow, 2000). What is needed now is to determine whether p-Erk plays a role in both phase shifting and in output pathways in the same cellular clock system.

Joseph C. Besharse
Department of Cell Biology, Neurobiology, and Anatomy
Medical College of Wisconsin
Milwaukee, Wisconsin 53226

Selected Reading

Besharse, J.C., and Iuvone, P.M. (1983). Nature *305*, 133–135.

Cahill, G.M., and Besharse, J.C. (1993). Neuron *10*, 573–577.

Cahill, G.M., and Besharse, J.C. (1995). Prog. Retinal Eye Res. *14*, 267–291.

Dunlap, J.C. (1999). Cell *96*, 271–290.

Ginty, D.D., Kornhauser, J.M., Thompson, M.A., Bading, H., Mayo, K.E., Takahashi, J.S., and Greenberg, M.E. (1993). Science *260*, 238–241.

Hasegawa, M., and Cahill, G.M. (1999). J. Neurochem. *72*, 1812–1820.

Hsu, Y.-T., and Molday, R.S. (1993). Nature *361*, 76–79.

Ko, G.Y.-P., Ko, M.L., and Dryer, S.E. (2001). Neuron *29*, this issue, 255–266.

Molokanova, E., Trevedi, B., Savchenko, A., and Kramer, R.H. (1997). J. Neurosci. *17*, 9068–9076.

Obrietan, K., Impey, S., and Storm, D.R. (1998). Nat. Neurosci. *1*, 693–700.

Pierce, M.E., Shesberadaran, H., Zhang, Z., Fox, L.E., Applebury, M.L., and Takahashi, J.S. (1993). Neuron *10*, 579–584.

Roenneberg, T., and Merrow, M. (2000). J. Biol. Rhythms *14*, 449–459.

Sanada, K., Hayashi, Y., Harada, Y., Okano, T., and Fukada, Y. (2000). J. Neurosci. *20*, 986–991.

A System of Multimodal Areas in the Primate Brain

The primate cerebral cortex has traditionally been divided into separate territories for vision, touch, audition and movement. These functions are known to overlap in many parts of cortex, but until recently the regions of overlap were not well studied. In this issue of *Neuron*, Bremmer et al. (2000) report a major advance in understanding at least one set of areas in the human brain in which the senses are integrated. This finding joins a growing set of work in monkeys and humans on the integration of the senses with each other and with the control of movement.

Vision, touch, and audition converge in many areas of the monkey brain, including the deep layers of the